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Infectious Bronchitis

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Summary

Agent, Infection, and Disease. Infectious bronchitis is caused by the avian coronavirus, infectious bronchitis virus (IBV), which is found worldwide. Infections, depending on the strain, may cause an acute upper-respiratory tract disease, drops in egg production, decreased egg quality, and nephritis. The virus is transmitted by inhalation or direct contact with contaminated objects and morbidity is usually 100%; whereas, mortality can vary depending on a variety of host factors and the strain of the infecting virus. Chickens are the primary host but the virus has also been found in pheasants and peafowl. There exist many different antigenic types of the virus and due to a high rate of mutation, new antigenic variants constantly emerge. As a general rule, different antigenic types and variants have little to no cross-protection.

Diagnosis. The preferred diagnostic test is molecular detection of the viral spike (S1) gene or virus isolation in embryonating chicken eggs. Multiple antigenic types of the virus are identified by sequence analysis of the S1 gene or by the virus neutralization test using serotype-specific antibodies. The disease can also be diagnosed by demonstrating rising antibody titers against IBV between preclinical and convalescent sera.

Intervention. Attenuated live and killed vaccines are used in an attempt to control the disease. However, multiple different antigenic types and constantly emerging new types that do not cross-protect make it difficult to prevent transmission and disease.

Introduction

Definition. Avian infectious bronchitis (IB) is an economically important, highly contagious, acute, upper-respiratory tract disease of chickens and other fowl, caused by the avian gammacoronavirus infectious bronchitis virus (IBV). The virus is found worldwide and is transmitted by inhalation or direct contact with infected birds or contaminated litter, equipment, or other fomites. Vertical transmission of the virus within the embryo has not been reported, but virus may be present on the shell surface of hatching eggs via shedding from the oviduct or gastrointestinal tract. A poor viral polymerase proofreading mechanism resulting in genetic mutations and genome recombination events can lead to the emergence of new serotypes of the virus, which do not cross protect, complicating control by vaccination.

Avian coronaviruses similar to IBV also have been found in pheasants (*Phasianus colchicus*) and peafowl (Galliformes) and IBV-like viruses have been isolated from turkeys, teal (*Anas crecca*), geese (Anserinae), pigeons (Columbiformes), guinea fowl (*Numida meleagris*), partridge (Alectoris), and ducks (Anseriformes) (28). The turkey coronaviruses (TCoV) are described in Chapter 12. Gammacoronaviruses similar to IBV have also been detected in wild birds (71, 92, 138).

Economic Significance

The disease is characterized by respiratory signs, reduced weight gain, and reduced feed efficiency in meat-type broiler chickens infected with the virus. Infection also predisposes broilers to secondary opportunistic bacterial infections that can result in airsacculitis, pericarditis, and perihepatitis. Morbidity is almost always 100%, but mortality can vary depending on the age and immune status of the birds, the strain of the virus, and if secondary bacterial or viral pathogens are involved. Some strains of IBV are nephropathogenic and can cause high mortality due to kidney failure in susceptible birds.

In layer and breeder chickens, infection may result in reduced egg production of up to 70% and declines in eggshell quality. The virus can replicate in the oviduct and cause permanent damage in young hens resulting in limited egg production over a prolonged period of time and birds that fail to come into production (false layers). Eggs

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from breeds with pigmented shells may become pale, and the albumen can have a watery viscosity. Egg production often recovers but may be permanently depressed in flocks with no immunity to the virus.

Public Health Significance

Infectious bronchitis has no known human health significance.

History

Infectious bronchitis was first observed in the United States in North Dakota in 1930, and the first documented description of the disease was published by Schalk and Hawn in 1931 (160). Early descriptions of the disease were consistent with a mild form of infectious laryngotracheitis (ILT) (23), but in 1936, Beach and Schalm using neutralization studies in chicks showed that the virus that caused IB was different from the virus that causes ILT (11).

An important discovery occurred in 1937 when Beaudette and Hudson (13) found that IBV could be propagated in the allantoic cavity of embryonating eggs. In 1941, Delaplane and Stuart (69) suggested that IBV propagated in embryonating eggs might have immunizing value, which led to the first IB vaccine report by van Roeckel et al. (177). The first IBV vaccine in the United States was developed using the van Roekel M41 strain, which is a Mass serotype virus isolated at the University of Massachusetts, Amherst, in 1941. More on the history of the M41 type vaccines used in the United States and other Mass-type vaccines can be found here (96).

Another important discovery relating to the control of the virus was made by Jungherr in 1956 when he reported that an IBV isolated in Connecticut did not cross protect chickens against challenge with the original Mass isolate (108). This led to the awareness that different serotypes of the virus existed and that they did not cross protect.

In the 1960s, it was discovered that IBV could interfere with growth of Newcastle disease virus (NDV) in embryonating eggs and cell culture, which was significant because IBV and NDV vaccines are often given together (12, 150). Also in the 1960s, Winterfield and Hitchner reported that some strains of IBV can cause a nephritis-nephrotic syndrome, and the nephropathogenic strains Gray and Holte were isolated (181).

A significant advancement in the diagnosis of IBV occurred in the 1990s when several laboratories began identifying the type of IBV using molecular techniques (101, 111, 116, 123). This allowed for the rapid identification of many isolates and the comparison of viruses around the world. More about the early history of IB research can be found in the review by Fabricant (73) .

Etiology

Classification

Infectious bronchitis virus is a gammacoronavirus in the subfamily Coronavirinae and family Coronaviridae (https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/222/coronaviridae). The Coronaviridae family includes two subfamilies, Coronavirinae and Torovirinae, and within the Coronavirinae subfamily there are four genera, alphacoronavirus, betacoronavirus, deltacoronavirus, and gammacoronavirus. Alphacoronaviruses and betacoronaviruses are mammalian viruses. The deltacoronaviruses include species from wild birds and gammacoronaviruses include avian coronaviruses IBV and TCoV, as well as coronaviruses isolated from pheasant, goose, pigeon, and duck (28, 104). The Beluga whale coronavirus SW1 is also a gammacoronavirus (131). Additional coronaviruses, which are not assigned to a genus, include viruses isolated from an Asian leopard cat and Chinese ferret badger (72, 183).

Morphology

Infectious bronchitis virus is an enveloped virus with a round to pleomorphic shape. The virus particles are approximately 120 nm in diameter with club-shaped surface projections (spikes) about 20 nm in length (Figure 4.1), which gives the virus a crown-like appearance and hence the name corona (Latin for crown).

Infectious bronchitis virus strains differ in their density in sucrose gradients; particles with a full complement of

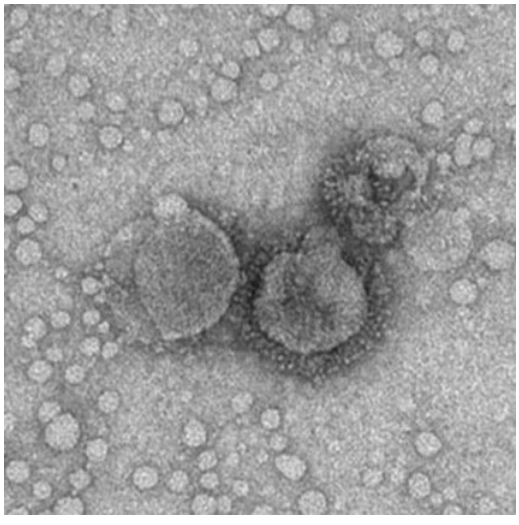


Figure 4.1 Virion of avian infectious bronchitis virus (IBV) illustrating club-shaped projections (Mark Jackwood, Department of Population Health, College of Veterinary Medicine, University of Georgia).

spikes have a density of 1.18 g/mL, and lesser-spiked particles may be as low as 1.15 g/mL.

Chemical Composition and Structure

The viral genome is a single-stranded positive-sense strand of RNA that is approximately 27.5–28 Kb in length. It is 5′ capped and has a poly-A tail at the 3′ end. The virions are made up of spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural proteins (148). The S glycoprotein is a trimer made up of two subunits, S1 and S2 (approximately 520 and 625 amino acids, respectively). The spike glycoprotein is a type I membrane protein containing a receptor binding domain, cleavage site, precoil domain, fusion peptide, heptad repeat regions, interhelical domain, transmembrane domain, and cytoplasmic tail (115, 148). It is involved in host cell attachment as well as virus and cell membrane fusion and entry into the cell (27, 180). Virus-neutralizing and hemagglutination-inhibiting (HI) antibodies are directed against the first third of the NH_3 end of the spike (32, 93, 115). The E protein is a small integral membrane protein involved in assembly of the virus (159). The M glycoprotein spans the viral envelope three times with a portion of the NH_3 end exposed on the outer surface of the virus. The N phosphoprotein encapsidates the viral RNA genome to form the helical nucleocapsid within the virion and interacts with the M and E proteins for virus assembly (102).

Virus Replication

For an overview of coronavirus replication see (75). The gene organization is 5′ untranslated region (UTR)-leader-1a/1ab-S-3a-3b-E-M-5a-5b-N-3′ UTR (Figure 4.2). The UTRs interact with viral encoded polymerase proteins and possibly host cell proteins for viral RNA transcription and replication (122). The open reading frames (ORFs) 1a and 1ab encode polyproteins that are post-translationally cleaved into 15 nonstructural proteins (Nsps) by a papain-like protease (PLP) and the main protease (Mpro), both of which are encoded on the polyprotein itself. The Nsps are involved in regulation of host cell functions and make up the viral RNA-dependent RNA polymerase (reviewed in [148]). Most coronaviruses have 16 Nsps but IBV as well as other gammacoronaviruses lack Nsp 1. The 1ab protein is translated through a -1 frame-shift at a "slippery" heptanucleotide sequence that is just upstream of an RNA pseudoknot structure. The 3a, 3b, 5a, and 5b ORFs are nonstructural proteins with largely unknown function. The virus replicates in the cytoplasm of the host cell and produces a 3′ co-terminal nested set of 5 subgenomic messenger RNAs. Each mRNA has a 5′ leader sequence that is joined to the mRNA during transcription. Although some of the subgenomic mRNAs are polycistronic, for the most part each mRNA encodes the protein at the extreme 5′ end. The full-length genome (mRNA1) encodes the viral polymerase polyproteins. Subgenomic mRNAs 2, 3, 4, 5, and 6 encode S, 3a/3b/E, M, 5a/5b, and N, respectively.

The S glycoprotein mediates host cell attachment, virus and cell membrane fusion, and entry into the host cell. Once in the cell, the viral genome acts as mRNA encoding Nsps 2 to 16, which forms to create the viral polymerase in double membrane vesicles at the Golgi (86). Transcription of viral subgenomic RNA and subsequent translation of viral proteins occurs in the cytoplasm. The S, E, and M proteins are inserted into the Golgi membrane, whereas the N protein binds to the newly synthesized viral genome to form the nucleocapsid. Interactions between nucleocapsid and E and M proteins result in budding of the virus particles at the cytoplasmic surfaces of the endoplasmic reticulum. The virus particles are transported to the plasma membrane in vesicles where fusion occurs to release the virus particles from the cell. Virus particles can also be released via cell lysis.

Susceptibility to Chemical and Physical Agents

Centrifugation forces of greater than 100,000 xg can sometimes result in the loss of spikes, or at least the S1 subunit. Incubation at 37 °C also can result in the loss of the S1 subunit, which is noncovalently attached to the S2 subunit by disulphide bonds (166).

Thermostability

Coronaviruses are heat liable, being inactivated after 15 minutes at 56 °C, but samples containing protein should be treated at 60 °C for at least 30 minutes to completely inactive the virus (149). Long-term storage of IBV is recommended at –80 °C. Survival up to 12 days and for as long as 56 days when ambient temperatures are below freezing has been reported. Thermo-instability requires that a cold chain always be maintained for samples sent to the laboratory for diagnosis.

Figure 4.2 Organization of the infectious bronchitis virus (IBV) genome.

Lyophilization

Infectious allantoic fluid lyophilized, sealed under vacuum, and stored in a refrigerator has remained viable for at least 30 years (reviewed in 96). Attenuated vaccines can be lyophilized in the presence of sucrose or lactose to preserve potency and extend shelf life.

pH Stability

The reduction in titer, following extremes of pH, are variable depending on virus strain. A pH 3 treatment at room temperature for 4 hours resulted in reductions in titer of 1–2 log10 for most isolates, but up to 5 log10 for others. Infectious bronchitis virus in cell culture was more stable in medium at pH 6.0 and 6.5 than at pH 7.0 to 8.0 (reviewed in [96]).

Chemical Agents

Infectious bronchitis virus, being an enveloped virus, is sensitive to ether, 50% chloroform, and 0.1% sodium deoxycholate (4°C for 18 hours). Most common disinfectants used in the poultry house inactivate IBV and no one type is recommended over another. The area to be disinfected should be free of organic material and disinfectants should be used at the manufacturer's recommended concentration. Treatment with 0.05% or 0.1% beta-propiolactone (BPL) or 0.1% formalin eliminated IBV infectivity. Only BPL treatment had no adverse effect on IBV hemagglutination (HA) activity, making it a good choice for creating HA antigen and killed vaccines.

Strain Classification

Many methods are used to differentiate and classify isolates of IBV, and they have been thoroughly compared (62). However, serotype and genetic typing, based on the sequence of the S1 protein, are most commonly used to classify strains. Serotype classification involves treatment of the virus with neutralizing antibodies, whereas genetic type classification involves examining the sequence of the S1 protein. Although not a hard and fast rule, strains of the virus that have greater than 90% amino acid similarity in the S1 gene (genetic type) are likely to be serologically related (serotype). Classification of virus types by immunization and challenge of birds, referred to as protectotype has also been reported (46).

There has been a lack of standardization of IBV strain nomenclature in the past, but most scientists have adopted the system suggested by Cavanagh in 2001 (29), which is similar to that used for avian influenza viruses. Basically, IBV strains are identified by the following scheme: IBV/bird type/country of origin/genetic type or serotype/strain designation/year of isolation. See *Genetic Classification* below for genetic type and lineage designations GI to GIV). Often IBV and bird type (assuming the isolate is from a chicken) are dropped, but if the isolate is not from a chicken or the type of chicken (broiler, layer, breeder) is important it is included. Examples of some viruses are US/GI-9,Ark/ArkDPI/81, US/GI-1, Mass/Mass41/41, Italy/GI-21,Italy-02/497/02, China/GI-19,LX4/QX/99, and IBV/Pheasant/UK/24/B171-3/99.

Serotype Classification

Traditionally, IBV serotypes have been defined by 2-way cross-virus neutralization (VN) testing in embryonating specific pathogen free (SPF) chicken eggs (81). This typing method involves reacting the unknown virus with antisera against known strains; then serotype-specific antibody is prepared against the unknown virus and reacted with known virus strains. The data are used to calculate a relatedness value using the Archetti and Horsfall formula (7).

Strain classification by the HI test has also been used, but most IBV strains do not spontaneously hemagglutinate and must be treated with neuraminidase (158). The HI antibody response following a single exposure of the virus can be highly strain specific, and the specificity and limited cross-reactivity of the early immune response are the basis for serotyping isolates using HI tests. However, multiple exposures to the virus, which is common in vaccinated birds, results in high and variable cross-reactions making it difficult to clearly differentiate strains using the HI test (45, 78).

Genetic Classification

Currently, most laboratories use nucleic acid approaches to characterize IBV isolates by genetic type (97, 112, 121). Typically, the reverse transcriptase-polymerase chain reaction (RT-PCR) is used to amplify the S1 gene or the hypervariable region of the S1 gene, followed by nucleic acid sequencing (112, 120) or less frequently, restriction fragment length polymorphism (RFLP) analysis (116, 123). The sequence of the whole genome is available for many isolates (genotype), and are available from Genbank (www.ncbi.nlm.nih.gov) for strains all over the world.

The deduced amino acid sequence of the S1 protein should be used to genetically type IBV isolates. The hypervariable region sequence of the S1 protein can be used to identify field viruses in a diagnostic laboratory setting, but generally is not sufficient for thorough characterization of genetic type. Analyzing the S1 gene phylogeny of 1286 IBV strains, Valastro et al. (175) defined 6 genetic types (GI to GVI) comprising 32 IBV lineages worldwide. The genetic type GI contains 27 different IBV lineages whereas GII to GVI each contain 1 IBV lineage.

There exists a correlation between percentage of similarity between S1 protein sequences and cross-protection (64). Generally, viruses that fall into the same

genetic type are related serologically, but exceptions do exist. Location of the amino acid differences (31) and sequence analysis of VN-monoclonal-antibody escape mutants (110, 137) indicate that a minimum number of changes can affect the conformationally-dependent neutralizing epitopes on the S1 protein resulting in little or no cross protection.

Strains of IBV and Viral Evolution

It is well known that a number of different types, subtypes, and variants of IBV exist, which is due to a high degree of genetic diversity that occurs through a high mutation rate and recombination events. Mutations include substitutions, which are the result of a high error rate and limited proofreading capability of the viral RNA-dependent RNA polymerase (RDRP), as well as insertions and deletions, caused by recombination events or by RDRP stuttering or slippage. Although IBV (and other coronaviruses) has a 3′ to 5′ exoribonuclease (exon) domain in Nsp 14 that is involved in proofreading and repair (132), the average rate of synonymous mutation is still high at approximately 1.2×10^{-3} substitutions/site/year (87, 89). Recombination has been reported in many coronaviruses including IBV (88, 167, 186). Since the replicase gene was shown to be a determinant of pathogenicity (8), recombination in the 1a/1ab genes associated with the RDRP can affect pathogenicity. Because the S glycoprotein gene is involved in host cell attachment (cell tropism) and contains viral neutralizing epitopes, recombination in the S glycoprotein gene can result in the emergence of new strains or serotypes of the virus as well as new viruses capable of causing disease in other host species (95). New IBV types, subtypes, and variants, whether the result of mutations, recombination, or both, continue to emerge, making control of IBV extremely challenging (96).

Laboratory Host Systems

Chicken Embryos

Infectious bronchitis virus grows well in 8 to 11 days of incubation in SPF chicken embryonating eggs following the inoculation of the allantoic cavity. The maximum virus titer in allantoic fluid (AF) is reached 1–2 days postinoculation (PI), although this peak can be delayed for non-egg-adapted field strains (62). For isolation of non-egg-adapted field strains, several sequential passages may be required to achieve high titers of virus in the AF. The extent of changes to the infected embryos that are induced by IBV vary greatly and are strain, dose, and age of the embryo dependent. Inoculation of 8-day-old embryos results in more extensive lesions and mortality than the same inoculation at 10–11 days of age. Characteristic lesions such as stunting (dwarfing) and curling of the embryo and its feet (125) occur with increasing passage as does the incidence of embryo mortality, which for an embryo-adapted strain can be observed as early as 2–3 days PI. Upon opening the air cell end of the egg, the embryo is seen curled into a spherical form with feet deformed and compressed over the head and with the thickened amnion adhered to it (Figure 4.3). A common internal lesion of the IBV-infected embryo is the presence of urates in the mesonephros of the embryonic kidney. This lesion is not pathognomonic for IBV infection and can also be observed in embryos infected with avian adenovirus. Confirmation of the presence of IBV antigen or viral RNA in inoculated eggs is therefore preferably performed 2–3 days PI and independent from the occurrence of embryo lesions.

Microscopic lesions in embryos infected with the IBV-M41 strain have been studied (125). Congestion with perivascular cuffing and some necrosis of the liver by the sixth day PI was observed. Lungs were pneumonic, characterized by congestion, cellular infiltration, and serous exudate in the bronchial sacs. In the kidney, interstitial nephritis with edema and distension of the proximal convoluted tubules and the presence of casts was noted. Glomeruli were not altered. The chorioallantoic membrane (CAM) and amniotic membrane were edematous. No inclusion bodies were observed.

Figure 4.3 Comparison of normal 16-day-old embryo (left) and curled, dwarfed, and infected embryo of the same age (right).

Cell Culture

Primary isolation of IBV field strains directly from pathological material in conventional monolayer cell cultures has proved unsuccessful (42). Adaptation of IBV strains is often necessary for sufficient replication leading to induction of cytopathic effect (CPE) (83). The number of passages that is needed for the adaptation can vary widely, even within the same serotype/genotype. Chick embryo kidney (CEK) cells and chicken kidney (CK) cells show the highest sensitivity for adapted IBV strains (126, 145). Chicken kidney cells form syncytia, which quickly round up and detach from the culture surface, appearing as large spheres with refractile contents. A few strains of IBV (e.g., the Beaudette strain) have been propagated successfully in the African green monkey Vero cell line, which has been used for many fundamental studies of IBV (56).

Organ Cultures

The propagation of IBV in organ cultures (OC) of trachea and other tissues has been reviewed (57). Tracheal organ cultures (TOCs) have proved very useful for the isolation, titration, and serotyping of IBV, because no adaptation of field strains is required for growth and the induction of ciliostasis. The sensitivity of TOCs for the detection of IBV strains is comparable to that of the use of embryonating SPF eggs. Following infection with IBV, ciliostasis, which is easily observed by low-power microscopy, usually occurs within 3–4 days (62). The presence of IBV in field samples must be confirmed by an IBV-specific test because ciliostasis also can be induced by many other agents.

Pathogenicity

Infectious bronchitis is primarily a disease of chickens with all ages being susceptible to infection. The pathogenicity of IBV can vary widely between strains. The clinical outcome of an infection in chickens depends on many variables such as the virus strain and type; sex and age of the chicken; immune status (vaccination, immune suppression, and maternally derived antibodies); coinfections; and environmental circumstances such as climate, dust, ammonia, and cold stress.

Infection is initiated via the respiratory tract regardless of the tissue tropism of the strain (respiratory, kidney, reproductive organs). The virus replicates and can produce lesions in many types of epithelial cells, including those of the respiratory tract (nasal turbinates, Harderian gland, trachea, lungs, and air sacs), kidney, and reproductive organs (oviduct, testes). Many strains also grow in many cells of the alimentary tract (esophagus, proventriculus, duodenum, jejunum, bursa of Fabricius, cecal tonsils, rectum, and cloaca) often with little pathobiological clinical effect (reviewed in 70).

All IBV strains produce lesions of varying severity in the respiratory tract depending on their virulence, chicken age at infection, genetic susceptibility of the chicken line, climate, and maternal or active immunity of the chicken. Therefore, IBV infections often increase the susceptibility to secondary respiratory infections or increase the damage of infections with primary respiratory pathogens. These increases have been shown for agents such as *Escherichia coli* (49, 85, 129, 130, 164), *Mycoplasma gallisepticum* (140, 170), *Mycoplasma synoviae* (73, 74, 90, 113, 119), *Mycoplasma imitans* (77), *Avibacterium paragallinarum* (152), Newcastle disease virus (139), and avian influenza virus (142). The damage caused by these secondary infections can be substantial, especially in broilers, resulting in a higher mortality, growth depression, increase of feed conversion, and higher condemnation rates. Proper vaccination against IBV also can be useful to prevent the chickens developing clinical IB and subsequent *E. coli* airsacculitis (129).

Although several strains of IBV are highly nephropathogenic, causing extensive and reproducible kidney disease in experimental conditions, many strains of IBV may be associated with nephritis to some degree in the field; environmental factors are probably important as to whether kidney complications are significant. The virulence of the strains for the kidney also depends on the age of infection. Young birds (less than two weeks of age) typically show more severe nephritis and higher mortality than older birds (1, 24, 188). Cumming (54) and Glahn et al. (84) enumerated some of the management factors that contribute to IB-related kidney disease. Greater mortality was seen in males, with cold stress, in certain breeds, increased levels of dietary calcium or when animal byproducts were the major component of high-protein diets. Some of these factors known to exacerbate the clinical disease have been used in experimental models to evaluate the clinical outcome of interaction between such factors and different IBV strains. Reddy et al. (154) reported significant differences between the replication kinetics of the nephropathogenic B1648 and respiratory M41 IBV strains when compared *in vitro* in respiratory mucosa explants and blood monocytes (KUL01(+) cells), and *in vivo* in chickens that might explain the different behavior of these strains in chickens.

Virulence for the reproductive tract also may differ among IBV strains. Presence of maternal antibody could prevent damage to the oviduct during an early-age IBV infection (21). In susceptible layers, different IBV strains produced a range of effects varying from shell pigment changes with no production drop to production drops of up to 70% (18, 19).

The virulence of IBV strains for other organs such as the alimentary tract seems to be low (reviewed in 70). However, several groups have reported outbreaks of a

"proventricular-type" IBV infection in chickens (179, 184) by QX and Q1 strains. Those studies did not show proof of local replication of the involved IBV strains, and the mortalities were exceptionally high for a solely IBV-induced disease. It remains unclear whether the reported signs were only caused by the IB strain or that another agent might be involved.

In recent years, coronaviruses have been detected in an increasing number of bird species and turkey, pheasant, goose, pigeon, and duck coronaviruses are considered avian coronaviruses in the gammacoronavirus with IBV. Mostly, the strains were not isolated but were detected by RT-PCR and sequencing, and little to no clinical signs were observed in these birds. Consequently, the majority of the detected strains could not be inoculated into chickens to determine infectivity and virulence. An exception are the coronaviruses isolated from pheasants with respiratory and kidney disease. These viruses differ in gene sequence from IBV to an extent similar to that exhibited by different serotypes of IBV (34). When three coronavirus isolates from pheasants were inoculated into chickens, no signs of disease were observed (124), which led to pheasant coronavirus (PhCoV) being officially considered as a species distinct from IBV. The issue of the coronavirus species determination is thoroughly discussed here (33).

Pathogenesis and Epizootiology

Incidence and Distribution

Infectious bronchitis is distributed worldwide and many dozens of serotypes and genotypes have been detected in all continents except Antarctica (64, 94). The first IBV variants were detected in the 1950s in the United States (73); however, a retrospective study (103) has shown that IBV variants were already circulating in the US poultry industries in the 1940s. Several serotypes can cocirculate in a given region with some strains being detected on several continents, while others seem to be regional.

Natural and Experimental Hosts

As explained previously, it is now accepted that the chicken is not the only host for IBV, although it is possible that IBV only causes disease in the chicken.

Age of Host Commonly Affected

All ages are susceptible, but the disease is most severe in chicks, often causing some mortality, especially with nephropathogenic strains. As age increases, chickens become more resistant to the nephropathogenic effects, oviduct lesions, and mortality due to infection (2, 25, 38, 53).

Transmission, Carriers, Vectors

Infectious bronchitis virus is highly contagious and spreads rapidly among chickens in a flock (65, 128). The disease has a short incubation period: susceptible birds placed with recently infected chickens usually develop clinical signs within 24–48 hours.

Transmission may be by either inhalation or ingestion of infectious virus particles by direct contact between infected and susceptible birds; by indirect contact through aerosol droplets or feces; and by exposure to virus-contaminated fomites, such as clothing, shoes, tools, and so on. Aerosol generation from the respiratory tract is a significant mode of transmission because of high virus concentrations in the respiratory tract during the acute stage of the infection. The highest concentration of IBV can be detected in the trachea during the first 3–5 days PI. After this period, the virus titer in the respiratory tract drops rapidly and in the second week PI can already be below the level of detection, especially in birds with a certain level of protection (62). Most likely, transmission by aerosol is especially effective over short distances, such as within a flock or premises, because the enveloped virion is inactivated relatively quickly in the environment.

The virus is also excreted in the feces and in the uric acid from kidney. During the chronic stage of an IBV infection, virus can be more readily detectable in the intestinal tract (cecal tonsils or cloaca swabs) and for a longer time than in the respiratory tract (62). Several authors report a long-term recovery (2–7 months) of IBV from infected or vaccinated flocks (3, 4, 38, 141); others report a re-excretion of IBV following T cell suppression by cyclosporine (16, 70) or at onset of lay (105). Possible explanations of long-term isolations or re-excretion of an inoculated virus are continual cross-infection within infected or vaccinated flocks, continual excretion of the virus at levels usually below the detection levels of tests, reactivation after treatment with cyclosporin (16), or reinfection due to contact with the infected feces from the previous infection after a decrease in level of protection. The two main candidate sites mentioned in these reports for persistence are cecal tonsils and kidney. The phenomena of possible long-term excretion and re-excretion may result in flock-to-flock transmission by direct or indirect contact with contaminated litter, fomites, or personnel, but further study is needed to understand if this is a common source of transmission.

Vertical transmission does not seem to be relevant for IBV, although Cook (41) could reisolate the challenge virus after infection in laying SPF hens and cockerels for 2 weeks from semen, for 1–7 weeks from the vitelline membrane of the eggs, and even from a small number of hatched chicks. However, the implication of this last finding for the field remained unclear, because these

chicks developed no clinical signs, did not seroconvert, and were not protected against challenge.

In view of the recent discovery of IBV in species other than the chicken, it should be considered that other species of birds might not only be able to transport IBV mechanically but also may actively multiply IBV or be a source of IBV outbreaks.

Incubation Period

The incubation period of IB is dose dependent and can be as short as 18 hours for intratracheal inoculation and up to 36 hours for ocular application.

Clinical Signs

The nonspecific respiratory signs of IB in susceptible chicks are gasping, coughing, sneezing, tracheal rales, and nasal discharge. Watery eyes may be observed, and an occasional chick may have swollen sinuses. The chicks appear depressed and may be seen huddled under a heat source. Feed consumption and weight gain may be significantly reduced. In chickens, older than six weeks of age, the signs are usually less clear, and the disease may even go unnoticed unless the flock is examined carefully by handling the birds or listening to them at night when the birds are normally quiet. The severity of the respiratory signs is influenced by the quality of the climate, housing, kind of bird, strain involved, IB vaccination program, and presence of coinfections including secondary infections.

Broiler chickens infected with a nephropathogenic virus may appear to recover from the respiratory phase and then show signs of depression, ruffled feathers, wet droppings, increased water intake, and mortality (54). Young age, cold stress, breed of chicken, increased levels of dietary calcium, and high-protein diets containing animal byproducts as the protein source are predisposing factors for the development of clinical signs during an infection with a nephropathogenic strain (54, 114).

In laying hens, the respiratory signs can be absent or very mild even in cases of clear production drops and the production of eggs with pale, unpigmented shells. The severity of the production decline may vary from slight up to 70% (18, 19) and depends on factors such as the causative virus strain and level of immunity against that strain, the timing of infection within the period of lay, and by coinfections. Following IBV infection at the onset of production a more severe drop in total production of normally shelled eggs, an increase in the number of abnormally shelled eggs, and more lasting adverse effects on egg weight and internal egg quality were observed, in comparison with infection after peak production. With mild drops in production, a normal level of production can be restored in 1 or 2 weeks. With severe drops of production, 6–8 weeks may elapse before production returns to the preinfection level, but in some cases, this is never attained.

In addition to production declines, IBV infections can cause a range of effects on the egg quality varying from loss of shell pigment, shell quality (misshapen, thin, soft-shelled, and rough-shelled eggs, Figure 4.4), thin to watery albumen (Figure 4.5) in a fresh egg, and decreased hatchability. Flocks with false layers fail to reach the normal rate of lay, whereas the flock looks healthy, behaves normally, and produces good quality eggs. The peak of production can be as low as 35% of expected production values (21).

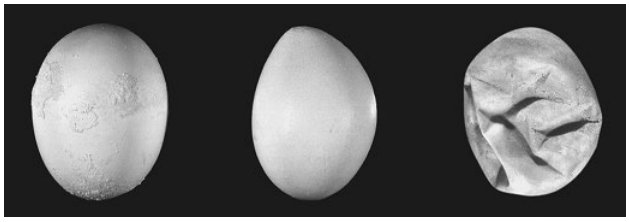


Figure 4.4 Thin-shelled, rough, and misshapen eggs laid by hens during an outbreak of IB. (Van Roekel)

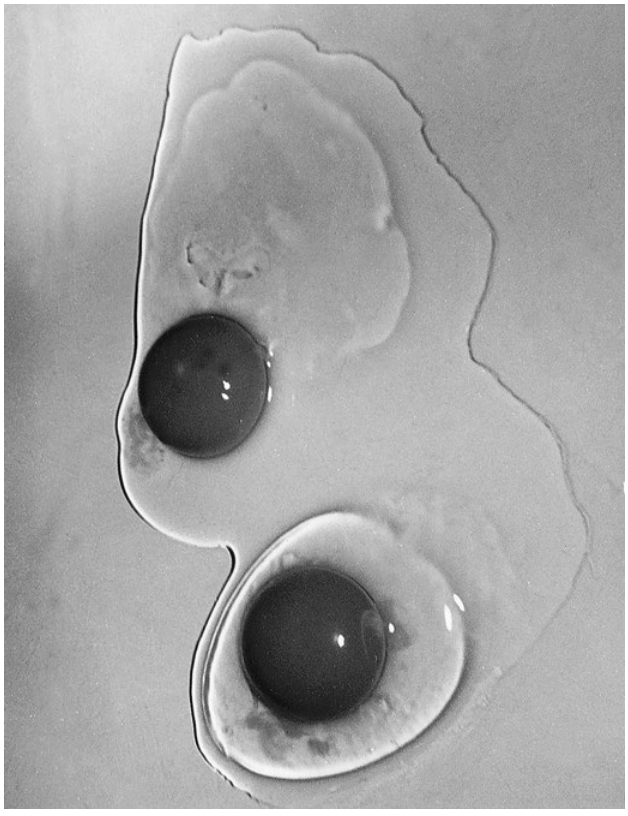


Figure 4.5 Contents of two eggs. Normal egg (bottom). Egg from chicken exposed to IBV at 1 day of age (top). Note watery albumen with yolk separated from thick albumen (51).

Pathology

Gross

Infected chickens have serous, catarrhal, or caseous exudate in the trachea, nasal passages, and sinuses. Air sacs may be foamy during the acute infection and then may become cloudy and contain a yellow caseous exudate. Areas of pneumonia may be observed around the large bronchi. Nephropathogenic infections may produce swollen and pale kidneys with the tubules and ureters often distended with urates (55, 187) (Figure 4.6).

Fluid yolk material may be found in the coelomic (abdominal) cavity of chickens that are in production, but this is also seen with other diseases that cause a marked drop in egg production. Cystic left oviducts may be a consequence of IBV infection of an unprotected bird at a young age (14, 21, 37, 52, 68, 106) and are a cause of false layers resulting in reduced peak in egg production when the flock reaches maturity. Effects of IBV infection on the reproductive tract of chickens in production have been detailed by Sevoian and Levine (163). They observed reduced length and weight of the oviduct in infected birds as well as regression of the ovaries.

Figure 4.6 Kidney lesions associated with infectious bronchitis (IB) caused by T strain of virus. Note swollen kidneys with tubules and ureters distended with urates (55).

Microscopic

The tracheal mucosa of chickens with IB is edematous. There is loss of cilia, rounding and sloughing of epithelial cells, and minor infiltration of heterophils and lymphocytes within 18 hours of infection. Regeneration of the epithelium starts within 48 hours but cilia recovery begins 7 to 8 days later. Hyperplasia is followed by massive infiltration of the lamina propria by lymphoid cells and the formation of a large number of germinal centers often forming after 7 days. If air sac involvement occurs, there is edema, epithelial cell desquamation, and some fibrinous exudate within 24 hours. Increased heterophils can be observed later with lymphoid nodules, fibroblast proliferation, and regeneration by cuboidal epithelial cells (155) (Figure 4.7A, B, C, D, E, F).

The histological changes post IB vaccination and challenge in the Harderian gland includes a sharp increase in the number of plasma cells, hyperemia, and extensive lymphoid follicle formation (59, 172).

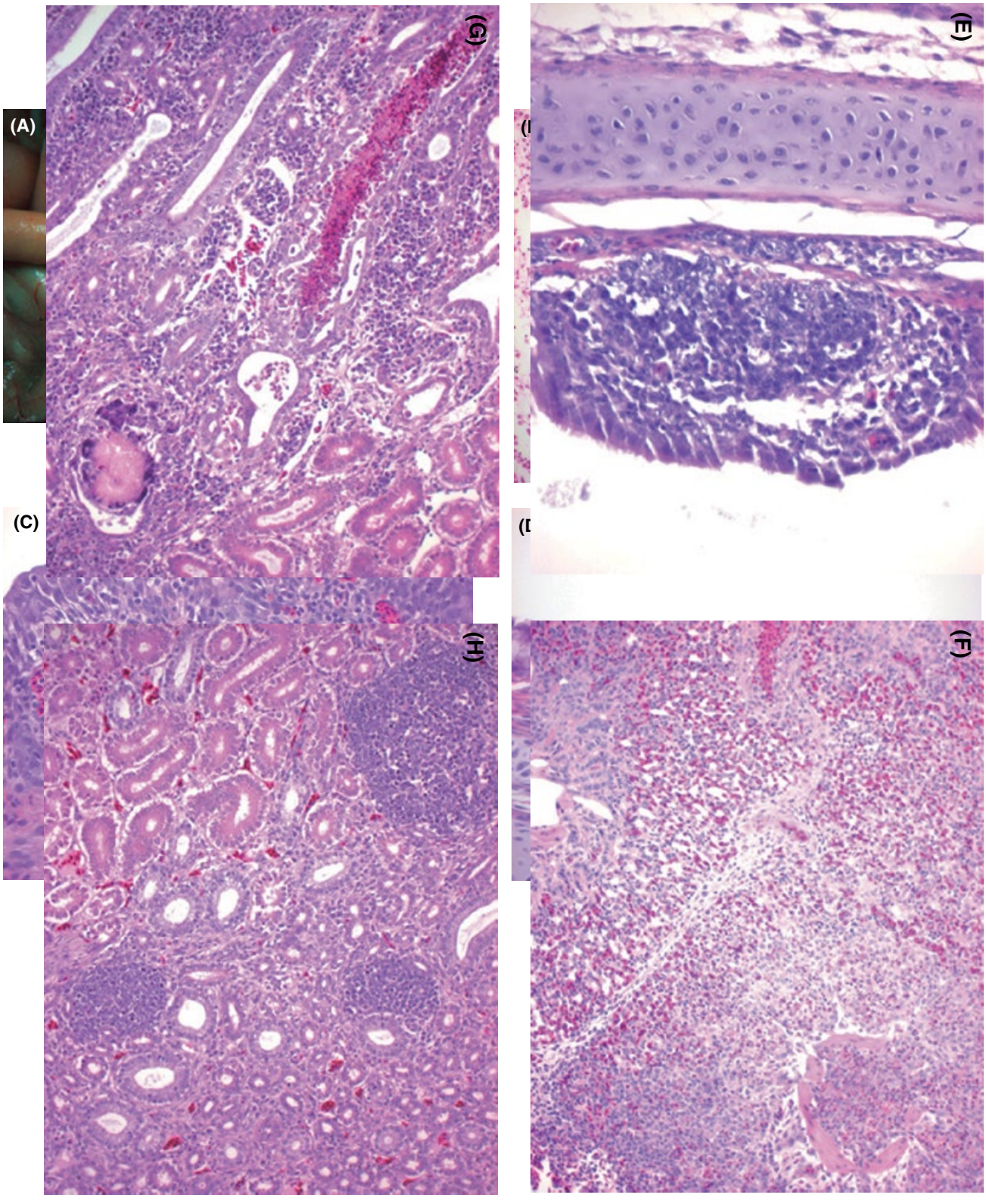
The kidney lesions of IB are principally those of an interstitial nephritis (Figure 4.7G and H) (35, 36, 155). The virus causes granular degeneration, vacuolation and desquamation of the tubular epithelium, and massive infiltration of heterophils in the interstitium in acute stages of the disease. The lesions in tubules are most prominent in the medulla. Focal areas of necrosis may be seen along with indications of attempted regeneration of the tubular epithelium. During recovery, the inflammatory cell population changes to lymphocytes and plasma cells. In some cases, degenerative changes may persist and result in severe atrophy of one or all of the divisions of the nephrons. In urolithiasis, the ureters associated with atrophied kidneys are distended with urates and often contain large calculi composed mainly of urates.

Experimental IBV infection of the oviduct of mature hens resulted in decreased height and loss of cilia from epithelial cells; dilation of the tubular glands; infiltration by lymphocytes, other mononuclear cells, plasma cells, and heterophils; and edema and fibroplasia of the mucosa of all regions of the oviduct (155, 163).

The histological lesions in the enteric tract appear to be mild for IBV infections that are free of coinfections, reviewed in (70). No lesions were detected in any part, from proventriculus to ileum, in chickens that had been infected with the enterotropic G strain; only the rectum showed desquamation of the cells from the tips of villi, congestion, and focal infiltration with lymphocytes, macrophages, and some heterophils (6). More recent reports of proventriculitis associated with IBV strains of the QX and Q1 genotype need confirmation of the role of IBV in these lesions (66).

Immunity

Aspects of immunity to IBV have been reviewed previously (30, 70). Although differences in both



breed- and strain-related genetic resistance to IBV infection have been described in chickens (10, 22, 47, 48, 144, 165), relevant data about the genetic resistance of commercial lines of chickens are not available.

Innate Immunity

The innate immune system is involved in directing the adaptive responses to IBV and can also act as expresser of the adaptive immunity (109). It has been shown that IBV induced a diversity of local innate effectors and Th1-based adaptive immunity during the early phase of IBV infection, and that these immune effectors are responsible for the rapid clearance of virus from the trachea (178). Mannose-binding lectin (MBL), an innate pathogen pattern-recognition molecule, is involved in the regulation of the adaptive immune response to IBV (109) and is able to bind specifically to the spike S1 protein of IBV and subsequently block the attachment of S1 to IBV-susceptible cells in chicken tracheal tissues (185). Recent work suggests that macrophages might play a role in spreading of the virus within the bird (5, 154).

Active Immunity

Cell-Mediated Immunity. Work on cell-mediated immune responses to IBV has been reviewed (70), and specifically for the cytotoxic T lymphocytes (CTL) (40). Work with the nephropathogenic Gray strain showed that the MHC-class 1 restricted CTL response correlated much better with the initial elimination of virus from lungs and kidneys during the acute phase of infection than the humoral IgM and IgG response (161). Adoptive transfer of different kinds of immune T cells to chicks prior to infection with the Gray strain demonstrated that IBV-primed CTLs with αβ T cell receptors (TCR2) could protect chicks from acute infection in the respiratory tract (162).

Humoral Immunity. Vaccination or infection with IBV results in an antibody response that can be detected using different kinds of group-specific and serotype-specific techniques. Usually, the first antibodies can be detected in serum and lacrimal fluid between one and two weeks PI. However, the level of antibody response depends on many variables including age at inoculation, presence and level of maternally derived antibodies, level of immunity at the time of vaccination/infection, application route, genetics, and immunosuppression, reviewed in (62). In serum, IgA, IgG (IgY), and IgM can be detected; in lacrimal fluid and tracheal washings, IgA and IgG are the commonly detected antibody classes. An important part of the IgA in the lacrimal fluid originates from the Harderian glands (9, 61). The IgG concentration in the same fluid is largely the result of passive transport of IgG from the serum (61, 171). IgM is only present for a few weeks after infection or vaccination; therefore, its detection is indicative of a recent exposure (62).

Serotype-specific virus neutralizing antibodies are induced by the amino-terminal S1 subunit of the S glycoprotein (31). In young birds, IBV ELISA and VN antibody levels in tears were not accurate indicators of IBV immunity as determined by challenge with Mass/Mass41/41. High tear IBV antibody titers were observed in some chickens determined to be susceptible to IBV challenge and low tear antibody titers were detected in some protected chickens (80). No correlation was found between the serum ELISA antibody titers and the degree of kidney protection against the nephropathogenic Belgium/B1648/96 strain (147). In another study, no antibody was detectable post vaccination in post hatch cyclophosphamide bursectomized chickens and still the birds resisted challenge (39). In laying birds, a clear correlation was found between the level of HI antibodies against the challenge virus and the level of protection against egg drop (18–20, 76) as long as these were the result of a live priming and subsequent boost using an inactivated vaccine.

Immunosuppression

Despite many studies that have detected neutralizing IBV serum and local antibodies post-vaccination, the relevance of antibodies and cellular immunity in the mechanism of protection against infection and disease is still largely unknown. Infections with virulent infectious bursal disease virus (IBDV) strains in birds at one to eight days of age prior to the IBV vaccination have shown to decrease the efficacy of an IBV vaccination, including a decrease in antibody response to the vaccination and longer excretion of the challenge strain postinoculation (146, 157, 182). A similar effect was reported after *in ovo* bursectomy in line C White leghorns. These birds experienced a more severe and longer lasting infection than the intact birds and also developed less respiratory protection against a secondary challenge (43). A US/Ark/Ark-DPI/81 challenge at 15 days of age in immunodeficient SPF birds by a combined chicken anemia virus and IBDV inoculation at 7 days of age resulted in more severe and persistent clinical signs and lesions, a delayed and reduced antibody response, and increased and persisting viral shedding (173).

Maternally Derived Immunity

High levels of maternally derived antibodies significantly reduced the extent of clinical signs or damage to trachea, kidneys, and oviduct due to IBV infection in chicks during the first days of life (21, 54, 58, 68, 134, 135). Several groups have reported a negative effect of high levels of maternal antibodies against the vaccine strain when it is applied on day of hatch, whereas others did not detect a

lower efficacy of the day-old vaccination in the presence of maternal antibodies (58, 60).

Diagnosis

Diagnosis of IB is based on the clinical history, lesions, seroconversion (rising IBV antibody titers), IBV antigen detection by a number of antibody-based antigen capture assays, virus isolation, and detection of IBV RNA (81). Thorough diagnosis of IBV includes identification of the serotype or genetic type of the virus so that appropriate vaccines can be used. The many approaches used to detect the virus or antibodies induced by it have been described and critically compared (62) .

Isolation and Identification of the Causative Agent

Although primarily a respiratory pathogen, IBV also can infect epithelial cells in the kidney, oviduct, and gastrointestinal tract. Knowledge of the pathogenesis of IBV, reviewed in (153), has been instructive for effective sample collection to detect the virus.

Virus Isolation

Tracheal swabs or fresh tracheal tissue is the preferred sample, especially within the first week of infection, and samples should constantly be kept cold (on wet ice) until tested. Titers of IBV reach a maximum in the trachea by day 3–5 PI, after which they decline rapidly. Because the virus initially grows in the upper respiratory tract and then spreads to non-respiratory tissues, kidney and cecal tonsils collected at postmortem examination can be of value in cases in which more than one week has elapsed since the start of infection. However, it should be recognized that vaccine viruses could also be found persisting in cecal tonsils. Although the virus can replicate in the gastrointestinal tract, cloacal swabs or fecal material are difficult samples from which to isolate the virus. Additionally, samples from the lung, kidney, and oviduct should be considered, depending on the clinical history of the flock (3).

When collecting samples from a large flock, both healthy birds and those with clinical signs should be sampled. Typically, clinical signs begin 3–5 days following infection when the virus is no longer at peak titer. In mild cases of the disease, clinical signs due to the virus may go unnoticed until secondary pathogens become involved, at which time IBV is no longer present. Alternatively, the placement of susceptible sentinel chickens has been described (78) and can be beneficial when direct sampling is unsuccessful.

Samples for virus isolation commonly are inoculated into the allantoic cavity of 9- to 10-day-old embryonating chicken eggs or TOCs, preferably from an SPF source. Fluids should be harvested after 48–72 hours from either culture system and passed at least 3–4 times before being called negative based on failure to cause lesions or death in embryos, or ciliostasis in TOCs (81). However, these observations are not in themselves sufficient to confirm the presence of IBV; the presence of the virus must be confirmed by VN, HI, immunofluorescence, immunohistochemistry, detection of the viral nucleic acid, or electron microscopy (15).

Coronaviruses similar to IBV in wild or domestic birds other than chickens may or may not replicate in embryonating chicken eggs (34, 104).

Confirmation and Typing of IBV by Antibody-Based Methods

Detection of IBV directly or indirectly in postmortem material (scrapings of tracheal mucosa or other tissues) or virus grown in embryonating eggs using serotype specific or monoclonal antibodies has been done but the results are not always easy to interpret, especially from direct field specimens because of nonspecific reactions (reviewed in [62]). All IBV serotypes appear to have common epitopes (group-specific antigens) likely due to the moderately high amino acid sequence identity within the N and M proteins and conserved regions of the S2 protein. Following a first infection with IBV, most of the antibody response is serotype-specific. A second infection, especially with a different serotype, results in a more broadly reactive serum. Because chickens in the field will almost certainly have been vaccinated (sometimes multiple times) with attenuated live vaccines (broilers and pullets) and killed vaccines (layers and breeders) against IB, field sera are not very useful for serotyping unknown viruses. Only sera induced experimentally (the procedure can be found in [81]) using SPF chickens should be used for determining the serotype of a virus.

The serotype of IBV has traditionally been determined by the VN or HI test (see Serotype Classification, above). However, virus-neutralization testing, whether conducted in embryonating eggs or in TOC, is time consuming and labor intensive, and the HI test suffers from nonspecific cross-reactivity. Thus, molecular-based tests have for the most part replaced VN and HI testing.

Confirmation and Typing of IBV by Nucleic Acid-Based Methods

The real-time RT-PCR test, also known as quantitative RT-PCR, is becoming more widely used to detect IBV directly from clinical samples (26, 98). Advantages of this test are that many samples can be examined in a short

period of time, it is cost-effective, and it gives an indication of the level of viral nucleic acid in the sample. Recently IBV type specific primers and probes for some strains of the virus have been developed for real-time RT-PCR IBV type specific testing (156).

Conventional RT-PCR also can be used to detect the presence of IBV nucleic acid in a clinical sample; however, passage in embryonating eggs is sometimes necessary to obtain a positive result. Identification of the type of IBV in the sample is determined by sequence analysis of amplicons from the S1 gene (see Genetic Classification, above). Advantages of genetic typing includes a rapid turnaround time and the ability to detect a wide variety of IBV types. The hypervariable region of the S1 gene can be used to genetically type IBV in a diagnostic laboratory setting but the entire S1 gene should be used for complete characterization. Spike sequence data can be used to identify any IBV type as well as previously unknown field isolates and variants and phylogenetic analysis of unknown field isolates and variants with reference strains can be used to establish relatedness (100, 112, 120, 136, 143, 176).

Serology

Demonstration of rising antibody titers against IBV between preclinical and convalescent sera can be used to diagnose IBV infection. Because the ELISA, immunofluorescence, and agar gel precipitan (AGP) tests all bind antibody to group-specific antigens, they cannot be used to differentiate serotypes. Currently the ELISA test is the most widely used serologic test for antibodies against IBV because it is inexpensive and can be used to test a large number of samples in a short time. Commercial tests are available and typically detect antibodies (IgG) after one week postinfection (67, 127, 133).

Routine serology also can be done with the AGP and HI tests, reviewed in (62). Although the AGP test can detect antibodies within the first week of infection, the strongest precipitating antibodies (IgM) are short-lived, and IgG is poorly reactive. Thus, the AGP test is not recommended for detection of antibodies beyond two weeks PI (67). Although cross-reactive antibodies can be detected in the HI test, sensitivity may suffer, because this is largely a serotype-specific reaction. Nonetheless, the low cost, simple equipment, and speed of the HI test makes it a useful procedure as long as the test limitations are considered.

Differential Diagnosis

The clinical presentation of IB may resemble mild forms of other acute respiratory diseases such as Newcastle disease (ND), ILT, low-pathogenicity avian influenza, avian metapneumovirus, and infectious coryza. Newcastle disease caused by velogenic viscerotropic or neurotropic strains of paramyxovirus type 1 produces much higher mortality than IBV. Lentogenic ND virus infections with pneumotropic strains and low pathogenicity strains of avian influenza like H9N2 produce mild to moderate respiratory disease with low mortality and, thus, may resemble IB. Infectious laryngotracheitis tends to spread more slowly in a flock, but respiratory signs may be more severe than with IB, and infectious coryza typically causes facial swelling that occurs only rarely in IB. Egg production declines and shell quality problems in flocks infected with ND virus, avian influenza virus, ILT virus, avian metapneumovirus, and *Avibacterium paragallinarum* (infectious coryza) as well as avian mycoplasmas and EDS adenovirus are similar to those seen with IBV, except that in the case of EDS adenovirus, internal egg quality is not affected.

Intervention Strategies

Management Procedures

Ideal management includes strict isolation, high biosecurity, and repopulation with a single age of chicks, following the cleaning and disinfection of the poultry house and equipment in contact with poultry or poultry litter and composting or removal of the feces from the premises. Because IBV is highly infectious, immunization is needed in many areas in an attempt to prevent production losses due to IB.

Vaccination

Chickens just recovered from infection or recently vaccinated are protected from challenge with the same virus (homologous protection), but the extent of protection against challenge with other IBV strains (heterologous protection) varies. Challenge of vaccinated birds with homologous virus results in much lower shedding of challenge virus, and for a shorter period, than in unvaccinated birds (50, 65, 118, 147).

Types of Vaccine

Both live and inactivated virus vaccines are used for IBV immunization. Live vaccines are used in meat type (broiler) chickens and for the initial vaccination and priming of breeders and layer pullets. Infectious bronchitis virus strains used for live vaccines are attenuated by serial passage in embryonating chicken eggs (17), sometimes in combination with heat treatment (99). Evidence that some vaccines increased in virulence after back-passage in chickens (91) demonstrates the potential for enhancement of virulence of such vaccines by contin-

ued circulation of the vaccine virus, a rolling infection, in a flock that is often caused by poor vaccination procedure, resulting in vaccination of only a fraction of birds in the house. The use of fractional doses of IB-attenuated vaccines has been associated with enhancing cyclic infections in a flock and an increase in vaccine-associated virulence.

The Mass/Mass41/41 strain, Mass/H120/55, and other vaccines of the Massachusetts serotype are commonly used in most countries. The goal of a vaccination program is to cover the antigenic spectrum of isolates in a particular country or region. When vaccinations with a single serotype are not providing sufficient protection against the prevailing field strains, vaccines of other serotypes can be added to the program. The broadening of the protection can be achieved by adding vaccines to the program that are homologous to the most important prevailing field strains (80, 81, 100, 104, 108) and/or by using combinations of vaccines that are able to induce a broad cross-protection against many strains (45, 63, 79).

Several studies have shown that live IBV, Newcastle, ILT, and avian metapneumovirus vaccines might interfere with each other's replication, humoral response, and induced level of protection when administered together or in relatively short intervals of each other (44, 79, 151, 168, 169, 174). The level and direction of interference is likely dependent on strains, dose, method and interval of application, maternally derived antibodies, and active immunity.

Inactivated oil-emulsion vaccines are administered to breeders and layers prior to the onset of egg production. Pullets may be vaccinated between 10 and 18 weeks of age depending on the immunization program. The efficacy of inactivated vaccines depends heavily on proper priming with live vaccine(s). Inactivated vaccines must be administered to birds individually, by intramuscular or subcutaneous injection. Inactivated vaccines induce high levels of serum antibody and increase protection to internal tissues, kidney, and reproductive tract (19, 20, 64, 117, 120). In contrast to live vaccines, inactivated vaccines are not nearly as effective at preventing infection of the respiratory tract following challenge with the homologous virulent virus (49).

New "variant" strains may be used to prepare inactivated autogenous vaccines for controlling IB in laying birds without the risks of using a live variant that could spread to and potentially cause disease in nearby flocks. Inactivated variant vaccines may offer better protection against challenge with the virulent live variant IBV than inactivated vaccines containing standard serotypes such as Mass and Conn (117).

Application Methods

Under experimental conditions, live vaccines are usually administered individually by eye drop or intranasal application. In the field, live vaccines are usually applied by the mass application methods including coarse spray, aerosol, or drinking water (107). The mass application of IBV vaccines in the field is known for its many variations in: (1) application technique (eye drop, coarse spray, drinking water, aerosol); (2) quantity, quality, and temperature of the water used to dilute the vaccine; (3) dosage; and (4) the combination of different vaccines (e.g., IBV with NDV vaccines). Many of these factors can have a negative effect on the efficacy of the vaccine under field conditions.

Despite the potential negative effect of the maternally derived antibodies on the efficacy of the vaccination in the first days of life (see Immunity), vaccination by spray application of maternally immune 1-day-old commercial chicks is efficacious and routinely performed, especially in the broiler industry. Besides the convenience, vaccination in a hatchery can be much better controlled than that in the poultry house.

Application by the drinking water system requires management measures to be taken to ensure that all birds can drink a sufficient amount of freshly prepared vaccine within a few hours, and should include the complete emptying of the water system before filling it with the vaccine. The water that is used with the vaccine should be of high quality, cold, and free of chemicals that can harm the vaccine such as sanitizers (many municipal sources of water contain chlorine, which can inactivate the vaccine). The incorporation of powdered skim milk at a 1:400 concentration or another suitable product has been shown to stabilize the virus titer during vaccine administration and resulted in better IBV and NDV immune responses in a field trial with 76 flocks (82).

The complexity of mass application methods can easily be underestimated resulting in decreased efficacy and undesired circulation of the vaccine through the flock. This might lead to an increased susceptibility to secondary bacterial infections (49, 85, 129, 130, 164) and reversion of the vaccine virus to virulence (91). As yet, no IB vaccines have been applied *in ovo*; all commercially available vaccines reduce hatchability to unacceptable levels.

There are probably dozens of serotypes/genotypes of IBV currently awaiting discovery, and IBV by nature is constantly changing through mutations and recombinations, which will pose challenges to the poultry industries and to vaccine developers. Given the current technology, it will only be economically feasible to develop new vaccines against a small number of new types of IBV. Therefore, control of IB will continue to involve "juggling" a small selection of vaccines, in combination with good management practices.

Treatment

No specific treatment exists for IB. Provision of additional heat to eliminate cold stress, good air quality,

elimination of overcrowding, and attempts to maintain feed consumption to prevent weight loss are flock management factors that may help reduce losses from IB. Treatment with appropriate antibiotics may be used to aid in reducing the losses from airsacculitis resulting from infection by secondary bacterial pathogens. In case of clinical nephritis, a decrease in protein levels in the food and a supply of electrolyte replacers in the drinking water might be helpful to compensate for the acute loss of sodium and potassium and thereby reduce mortality from nephritis. The recommended concentration for treatment is 72 mEq of sodium and/or potassium, with at least one-third in the citrate or bicarbonate salt form (54).

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